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Effects on the Fetal Rat Intestine of Maternal Malnutrition and Exposure to Nitrofen (2,4-Dichclorophenyl-p-Nitropenyl Ether)

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16. ABSTRACT

The effects of maternal protein-energy malnutrition and exposure to nitrofen on selected aspects of intestinal morphology and function were studied in the fetal rat. Pregnant rats were fed, throughout gestation, diets containing 24% or 6% casein as the sole source of protein. Reduced total food intake produced protein-energy malnutrition (PEM). Each diet group was divided in half and gavaged with either 12.5 mg nitrofen in corn oil/kg/day or corn oil carrier only from days 7 to 21 of gestation. Body weight, intestinal weight, length, and diameter were measured as were villus length (VL), villus width (VW), and number of villi per length of intestine (VMM). Protein (horseradish peroxidase) and lipid absorption were studied histochemically. Lactase and dipeptidase activities were determined in proximal, medial, and distal thirds of the intestine. Results showed that the restricted maternal diet resulted in reduced fetal body weight (BW), intestinal weight (IW) and length (IL), reduced IW/BW and IW/IL ratios, VH, and VMM. The VW was reduced only in the distal third. Protein and lipid absorption were unaffected. Lactase and dipeptidase activities were reduced. Maternal nitrofen exposure resulted in reduced body weight, intestinal size, and lipid absorption, with some evidence of interaction with the diet effects on enzyme activities. It is concluded that effects of maternal malnutrition were extensive, but that nitrofen exposure, at this dosage level, is not likely to contribute to the postnatal fetal mortality rate in either adequately nourished or malnourished rats.

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Effects on the Fetal Rat Intestine of Maternal Malnutrition and Exposure to Nitrofen (2,4-Dichlorophenyl-*p*-Nitrophenyl Ether)

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The effects of maternal protein-energy malnutrition and exposure to nitrofen on selected aspects of intestinal morphology and function were studied in the fetal rat. Pregnant rats were fed, throughout gestation, diets containing 24% or 6% casein as the sole source of protein. Reduced total food intake produced protein-energy malnutrition (PEM). Each diet group was divided in half and gavaged with either 12.5 mg nitrofen in corn oil/kg/day or corn oil carrier only from days 7 to 21 of gestation. Body weight, intestinal weight, length, and diameter were measured as were villus length (VL), villus width (VW), and number of villi per length of intestine (VMM). Protein (horseradish peroxidase) and lipid absorption were studied histochemically. Lactase and dipeptidase activities were determined in proximal, medial, and distal thirds of the intestine. Results showed that the restricted maternal diet resulted in reduced fetal body weight (BW), intestinal weight (IW) and length (IL), reduced IW/BW and IW/IL ratios, VH, and VMM. The VW was reduced only in the distal third. Protein and lipid absorption were unaffected. Lactase and dipeptidase activities were reduced. Maternal nitrofen exposure resulted in reduced body weight, intestinal size, and lipid absorption, with some evidence of interaction with the diet effects on enzyme activities. It is concluded that effects of maternal malnutrition were extensive, but that nitrofen

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exposure, at this dosage level, is not likely to contribute to the postnatal fetal mortality rate in either adequately nourished or malnourished rats.

Key words: nitrofen, protein-energy malnutrition, fetal rat intestine, lactase, dipeptidase, morphology

INTRODUCTION

Nitrofen (2,4-dichlorophenylether) was an herbicide useful for both pre- and postmergent control of a wide variety of annual grasses and weeds along rights of way and in the commercial production of grains and vegetables. It has been voluntarily withdrawn from use by the manufacturer. In this study, nitrofen is used as a prototype in the study of the effects of diphenyl ethers.

The general population may be exposed to residues remaining on food crops, while occupational exposure may occur through dermal contact or inhalation [1]. It has been suggested that nitrofen's toxic effects are produced by competing with ADP in the ATP-synthetase reaction, inhibiting energy transfer [2].

Nitrofen has an LD50 greater than 2,600 mg/kg in the adult [3]. In the case of exposure to nitrofen during pregnancy, however, the material may be more toxic. It is reported to be fetotoxic at doses as low as 12.5 mg/kg maternal body weight/day [4,5], and to have an LD50 in fetuses of 116 mg/kg/day, with day 11 of gestation as the most sensitive day [6].

Ambrose et al were the first to observe that exposure to nitrofen during pregnancy caused a decrease in pup survival after birth [3]. Kimbrough et al suggested that the probable cause of death was a disturbance in lung development [4]. Later, Stone, and Manson [5] and Costlow and Manson [6] suggested that a combination of cardiac malformation and diaphragmatic hernia prevented the successful transition to extrauterine life. Kavlock et al noted that, in offspring of nitrofen-exposed mice and rats, the harderian glands do not develop properly, lung growth and renal development and function are altered, reproductive function is deficient, and serum thyroxine (T_4) levels are reduced [7–11]. Finally, Manson et al concluded that nitrofen teratogenicity in the rat is mediated at least in part by alteration in maternal or fetal thyroid status [12].

Recent studies of interactions between malnutrition, toxicants, and other environmental factors have suggested that current toxicity testing in which factors are studied individually may be inadequate [13]. Marked differences in the toxicity of some pesticides when the animal is protein deficient are reported to occur [14–17], but little is known of the effect of maternal protein deficiency on pesticide toxicity in pregnancy. Therefore, an investigation was undertaken to compare effects of protein deficiency and chronic exposure to nitrofen. This paper is a report on one aspect of this investigation. It compares the effects on the fetal intestine of maternal protein deficiency and maternal nitrofen exposure. The intestine was chosen because previous work had demonstrated the effect of maternal protein deprivation on fetal intestinal enzymes [18–20], digestion, and absorption of lipid, protein, and amino acids [21, 22].

MATERIALS AND METHODS

Virgin female Sprague-Dawley rats were obtained from Bantin & Kingman, Inc. (Fremont, CA) and housed singly in stainless steel wire-bottom cages. Animal

rooms were maintained at constant temperature (22 ± 1°C) and light was controlled to give a 10-hour light 14-hour dark cycle. Animals were fed a pelleted rat chow (Purina, St. Louis, MO) until mated. Tap water was available ad lib. When the females weighed 180–200 g, they were bred overnight with normal proven Sprague-Dawley males on a one-to-one basis. Mating was determined by the presence of copulation plugs. The morning following mating was designated as day 0 of pregnancy. On day 0, each pregnant female was assigned to either a diet containing 24% casein (control) or a diet containing 6% casein (PEM) as the sole source of protein with an additional 18% glucose to make the two diets isocaloric. Details of the composition of the diet have been published previously [23]. Diets were fed ad libitum throughout gestation and water was freely available. Since protein-deprived animals reduce their food intake, the result is protein-enegry malnutrition (PEM).

Each diet group was divided into two subgroups. Rats in one subgroup of each pair were gavaged on days 7–21 of gestation with 25 mg/kg body weight/day of nitrofen (99% pure, Chem Service Inc., West Chester, PA) dissolved in 0.5 ml corn oil (Mazola Oil, Best Foods, Englewood Cliffs, NJ), and the other, with 0.5 ml corn oil carrier only.

For studies using histological methods, two pups from each litter, obtained by cesarean section on day 21 of gestation, were randomly chosen for studies of morphological development of the intestine and two each for studies of lipid absorption and protein absorption using histochemical methods. Following decapitation, intestines from the pyloric sphincter to the cecum were removed from the pups, and the mesentery was excised. Intestines were quickly divided into proximal, medial, and distal thirds. The tissue was then fixed in Bouin's solution for at least 8 hours. Each section of tissue was further divided into 2–3-mm lengths before dehydration and paraffin embedding. Sections at 6 μm were stained with hematoxylin and eosin. Midplane sections were used for measuring intestinal diameter, villus height (VH), villus width (VW), and the number of villi per millimeter (VMM) of intestine.

Intestinal diameter was measured in longitudinal sections cut through the midplane of the segment. Only segments in which no obvious distension was apparent and which contained no meconium were measured. Using a calibrated ocular micrometer, the segment was measured at the midpoint of its length from one serosal wall to the one opposite [24].

Villus height was determined for the largest villi in each segment by counting, from the villus base to the tip, the number of enterocytes covering the lateral wall. Width of the same villi in each segment was obtained by direct measurement using an ocular micrometer [24].

The numbers of villi per millimeter were obtained by direct counts of the number of villus bases present in a longitudinal section delimited by the length of a calibrated ocular micrometer [24].

Lipid absorption was studied using a solution of emulsified cottonseed oil (Lipomul, Upjohn Co., Kalamazoo, MI) and diluted 1:1 with water. Pups weighing over 4.0 g received 0.04 ml of solution and pups weighing less than 4.0 g received 0.02 ml of solution. The volume of solution was administered into the duodenum of anesthetized pups by means of a PE 10 cannula passed through a small incision in the stomach and through the pyloric sphincter. After waiting 1 hour to allow for absorption, the animal was killed by decapitation and its intestine removed. The caudal third of the duodenum and the length of the jejunum that was filled with the emulsion was

removed and placed into cold neutral buffered formalin for 12 hours. This tissue was then cut into 2–3-mm sections, washed, and embedded by placing the sections longitudinally in O.C.T. Compound (Miles Laboratories, Elkhart, IN) in a mold. The entire mold was frozen in a mixture of crushed ice and 80% ethanol for 2 minutes. Longitudinal cryostat sections were cut at 4 μm and stained with Oil Red O and examined for lipid absorption. The villi in each millimeter length of stained tissue was scored for the degree of absorption as follows: IV: heavy absorption in cells along the entire length of the villus; III: medium absorption with large amounts of lipid absorption in apical enterocytes with lesser amounts in lateral enterocytes; II: light absorption with smaller amounts of lipid absorption in all enterocytes; I: minimal absorption with almost no visible absorption of lipid. Values for each classification were expressed as percentages of the total amount of tissue observed for each animal. These percentage figures were then converted to arcsin values to permit further statistical evaluation [25, 26].

Horseradish peroxidase (Sigma Type II, Sigma Chemical Co., St. Louis, MO) was used as the model for the study of protein absorption [27]. Two pups from each litter were gavaged with 0.05 ml of 2% aqueous solution of horseradish peroxidase (HRP). One hour later the pups were killed by decapitation and their intestines removed. The middle third of the intestine was then removed and tissue was placed in cold 4% sodium cacodylate-buffered glutaraldehyde, fixed at 4°C overnight, and transferred to gum sucrose for 12 hours. The tissue was then embedded as previously described. Cryostat sections were cut at 4 μm and stained for HRP using the method of Schneeberger and Karnovsky [28]. Jejunal HRP absorption was evaluated for each millimeter length of stained tissue as previously described for lipid absorption.

For studies of dipeptidases, the pups were taken by cesarean-section on day 21 of gestation and weighed. Those with body weights falling within ± 1 SD of the mean weight for their litter were decapitated and the small intestines were immediately removed as previously described. After lengths were measured, intestines were divided into proximal, medial, and distal segment of equal length. These three segments were separately weighed and frozen in liquid nitrogen.

The activities of the following dipeptidases were determined according to the method of Josefsson and Lindberg [29]: L-alanyl-L-glutamic acid (AGP), glycyl-L-valine (GVP), and glycyl-L-leucine (GLP). Protein was measured using the method of Lowry et al [30]. Dipeptides and their corresponding amino acids were obtained from Sigma Chemical Co. (St. Louis, MO). The enzyme activities were expressed as changes in optical density/minute/mg intestinal protein.

For determination of lactase, tissue was obtained as described for the dipeptidase assay. Individual intestinal segments were homogenized in a mortar with liquid nitrogen. Lactase activity was determined using the method of Dahlquist [31]. Protein was again assayed according to Lowry et al [30]. Enzyme units were defined as the activity hydrolyzing 1 μmole of lactose per minute per gram protein.

All data were analyzed using two-way analysis of variance with interactions using the litter as the statistical unit [32]. For diet and toxin effects separately, p-values of 0.05 or less were considered significant. For interactions, p-values of 0.10 or less were considered significant, as proposed by Snedecor and Cochran [33].

RESULTS

Table I shows the effects of PEM and nitrofen exposure on fetal body weight. It demonstrates that body weight was reduced in pups of dams fed the 6% protein diet

TABLE I. Effects of Nitrofen Exposure* in the Protein-Energy-Malnourished Fetal Rat on Body Weight and Intestinal Size**

	Treatment[a]				Significance of difference: P		
	Carrier only		Nitrofen exposure				
	24% casein	6% casein	24% casein	6% casein	D	N	I
n	12	12	12	12			
Body weight (g)	5.31 ± 0.06[a]	3.39 ± 0.14[b]	4.93 ± 0.13[c]	3.12 ± 0.08[b]	<0.0001	<0.005	NS
Intestinal weight (mg)	129.58 ± 3.61[a]	65.67 ± 2.38[b]	109.00 ± 6.46[c]	61.50 ± 2.64[b]	<0.0001	<0.005	<0.05
Intestinal length (cm)	19.40 ± 0.33[a]	14.77 ± 0.48[b]	17.07 ± 0.59[c]	13.35 ± 0.28[a]	<0.0001	<0.0001	NS
Ratios							
Intestinal weight/ body weight (mg/g)	24.41 ± 0.65[a]	19.77 ± 0.48[b]	21.90 ± 0.80[c]	19.69 ± 0.51[b]	<0.0001	NS	<0.05
Intestinal length/ body weight (cm/g)	3.65 ± 0.057[a]	4.42 ± 0.18[b]	3.46 ± 0.065[a]	4.30 ± 0.081[b]	<0.0001	NS	NS
Intestinal weight/ intestinal length (mg/cm)	6.69 ± 0.17[a]	4.49 ± 0.22[b]	6.35 ± 0.21[a]	4.58 ± 0.11[b]	<0.0001	NS	NS

*25mg in oil/kg maternal body weight/day by gavage.
**D, diet; N, nitrofen; I, interaction; NS, not significant.
[a]Data are mean ± SEM. Numbers in a row not sharing a common superscript letter are significantly different.

($p < 0.0001$). Nitrofen exposure also resulted in a reduction in body weight in the control group and in the PEM group ($p < 0.005$). There was, however, no indication of a statistically significant interaction between the two treatments.

The intestinal weight was reduced in pups of PEM dams ($p < 0.0001$). The effect of nitrofen exposure was also to reduce intestinal weight ($p < 0.005$); however, the effect was greater in the control group than in the PEM group as indicated by the statistically significant interaction ($p < 0.05$).

Fetal intestinal length decreased in the fetuses of protein-deprived dams ($p < 0.001$). It was also decreased in fetuses of nitrofen-exposed dams in both diet groups ($p < 0.0001$). There was no interaction between the treatments.

Overall, these data show that body weight and intestinal weight and length are decreased in the young of protein-energy-deprived dams. Nitrofen exposure caused a reduction in all three parameters in the control-diet-fed groups. In the protein-deprived groups, only intestinal length was significantly reduced.

The ratio of intestinal weight to body weight was reduced in pups from the PEM group ($p < 0.0001$). As indicated by the significant interaction ($p < 0.05$), nitrofen exposure interacted with diet, resulting in a decrease in the ratio confined to the control-diet-fed group, but not affecting the PEM group. Intestinal length to body weight ratio was increased in the protein-deprived pups. Nitrofen exposure had no influence on the diet effects. Intestinal weight to intestinal length ratios were reduced in the PEM group, but nitrofen exposure had no effect.

The effects of PEM and nitrofen exposure upon intestinal morphology are illustrated in Table II. Intestinal morphology was more responsive to the dietary change than to toxin exposure. Because the prenatal rat develops in a cranial to caudal gradient, all proximal segment measurements were greater than the medial and distal segment measurements. Intestinal diameter in the proximal segment was unaffected by diet or toxin exposure. The young of PEM dams had significantly reduced ($p < 0.0001$) villus heights (VH) and numbers of villi/mm intestine ($p < 0.05$) in the proximal intestinal segments, but villus width (VW) was unaffected. Villus widths were greater in fetal intestines in the nitrofen-exposed group ($p < 0.0001$). Villus height and villi/mm intestine were not affected by nitrofen exposure.

In the medial sections, intestinal diameter was reduced in the fetuses of PEM dams ($p < 0.0002$) and was higher in nitrofen-exposed young ($p < 0.02$) as compared with those of the corresponding diet group given carrier only. There was, however, no statistical evidence of an interaction between the two treatments. Villus height was reduced significantly ($p < 0.0001$), as was the number of villi/mm intestine ($p < 0.02$) due to maternal PEM, but was unaffected by nitrofen exposure. Villus width was unaffected by either treatment.

In the distal segments of the intestine, PEM pups showed significantly reduced intestinal diameter ($p < 0.0001$), villus height ($p < 0.0001$), villus width ($p < 0.05$), and in the number of villi/mm of intestine ($p < 0.005$). Further reduction in villus height ($p < 0.05$) and numbers of villi/mm intestine ($p < 0.05$) was evident in nitrofen-exposed pups. Villus height was significantly lower due to nitrofen exposure in the control diet groups ($p < 0.05$), but not in the PEM diet groups.

The results of the study of lipid absorption, seen in Table III, showed a toxic effect but little diet effect. Heavy absorption (grade IV) was dominant in those animals in the groups not exposed to nitrofen, while those exposed to nitrofen had a predominance of medium (grade III) and light (grade II) absorption. Sections showing

TABLE II. Effects of Nitrofen Exposure* in the Protein-Energy-Malnourished Rat on Fetal Intestinal Morphology**

	Treatment[a]				Significance of difference[a]: P		
	Carrier only		Nitrofen exposure				
	24% casein	6% casein	24% casein	6% casein	D	N	I
Proximal							
n	7	7	5	5			
Intestinal diameter (cm)	1.01 ± 0.029[a]	0.93 ± 0.04[a]	1.03 ± 0.031[a]	0.97 ± 0.03[a]	NS	NS	NS
Villus height (No. of cells)	67.12 ± 0.99[a]	55.82 ± 2.80[b]	67.37 ± 1.28[a]	56.70 ± 1.96[b]	<0.0001	NS	NS
Villus width (mm)	0.0754 ± 0.0021[a]	0.0754 ± 0.0018[a]	0.0832 ± 0.0019[b]	0.0894 ± 0.0026[b]	NS	<0.0001	NS
Villi/mm intestine	16.39 ± 0.063[a]	14.15 ± 0.65[ab]	15.25 ± 1.05[ab]	13.96 ± 0.46[b]	<0.05	NS	NS
Medial							
n	6	9	5	6			
Intestinal diameter (cm)	0.751 ± 0.02[a]	0.61 ± 0.02[c]	0.85 ± 0.02[b]	0.69 ± 0.045[ac]	<0.0002	<0.02	NS
Villus height (No. of cells)	49.04 ± 2.36[a]	42.99 ± 1.81[b]	49.83 ± 1.31[a]	39.03 ± 1.81[b]	<0.0001	NS	NS
Villus width (mm)	0.0550 ± 0.0015[a]	0.0554 ± 0.0027[a]	0.0527 ± 0.0018[a]	0.0573 ± 0.0021[a]	NS	NS	NS
Villi/mm intestine	12.62 ± 0.82[a]	10.84 ± 0.85[ab]	12.33 ± 0.47[a]	10.30 ± 0.90[b]	<0.02	NS	NS
Distal							
n	8	8	6	6			
Intestinal diameter (cm)	0.64 ± 0.28[a]	0.48 ± 0.01[b]	0.62 ± 0.01[a]	0.48 ± 0.03[b]	<0.0001	NS	NS
Villus height (No. of cells)	38.21 ± 1.16[a]	28.69 ± 1.22[b]	34.30 ± 1.13[c]	27.63 ± 1.00[b]	<0.0001	<0.05	NS
Villus width (mm)	0.0564 ± 0.0011[a]	0.0510 ± 0.0008[b]	0.0538 ± 0.0015[a]	0.0511 ± 0.0011[b]	<0.05	NS	NS
Villi/mm intestine	11.21 ± 0.50[a]	8.67 ± 0.37[b]	9.20 ± 0.68[b]	8.29 ± 0.51[b]	<0.005	<0.05	NS

*25 mg in oil/kg maternal body weight/day by gavage.
**D, diet; N, nitrofen; I, interaction; NS, not significant
[a]Data are mean ± SEM. Numbers in a row not sharing a common superscript letter are significantly different.

TABLE III. Effects of Maternal Nitrofen Exposure* and Protein-Energy-Malnutrition on Fetal Intestinal Lipid Absorption**

	Treatment[a]				Signficicance of difference[a]: P		
	Carrier only		Nitrofen exposure				
Grade	24% casein	6% casein	24% casein	6% casein	D	N	I
IV	73.47 ± 7.81[a]	55.50 ± 6.78[ab]	31.64 ± 9.85[b]	30.30 ± 11.38[b]	NS	0.002	NS
	(2.14 ± 0.18)[a]	(1.69 ± 0.15)[ab]	(1.12 ± 0.23)[b]	(1.06 ± 0.33)[b]	NS	0.002	NS
III	25.08 ± 7.67[a]	36.70 ± 7.30[ab]	49.64 ± 8.51[b]	38.60 ± 4.90[ab]	NS	NS	NS
	(0.96 ± 0.17)[a]	(1.27 ± 0.16)[ab]	(1.54 ± 0.19)[b]	(1.34 ± 0.14)[ab]	NS	NS	NS
II	1.45 ± 1.45[a]	7.80 ± 4.07[ab]	18.44 ± 3.32[bc]	31.10 ± 11.69[c]	NS	0.001	NS
	(0.62 ± 0.06)[a]	(0.42 ± 0.18)[a]	(0.83 ± 0.09)[b]	(1.09 ± 0.29)[b]	NS	0.0001	NS
I	0.00[a]	0.00[a]	0.28 ± 0.028[a]	0.00[a]	NS	NS	NS
	(0.00)	(0.00)	(0.02 ± 0.02)	(0.00)	NS	NS	NS
Total score	11.60 ± 0.17[a]	11.52 ± 0.13[ab]	10.84 ± 0.33[ab]	10.48 ± 0.76[b]	NS	0.03	NS

*25 mg in oil/kg maternal body weight/day by gavage.

**D, diet; N, nitrofen; I, interaction; NS, not significant

[a]Data are mean ± SEM, percent of total length. Numbers in a row not sharing a common superscript letter are significantly different. Arcsin transformations are given in parentheses.

minimal absorption (grade I) were rare, and only a few sections (0.28%) of the sections in the 24% casein-plus-nitrofen group showed no absorption. The total scores for nitrofen-exposed pups were reduced ($p < 0.03$). On the other hand, diet had no statistically significant effect.

There were no differences in absorption of horseradish peroxidase as a result of either diet or nitrofen exposure. Therefore, detailed data are not given.

The results of the study of activities of three dipeptidases are given in Table IV. Fetal intestinal GLP activity was reduced as an effect of diet in the medial ($p < 0.01$) and distal ($p < 0.0001$) portions, while maternal toxin exposure had no effect.

The effects of the treatments on GVP activity are more complex. There was no significant effect of diet in the carrier-treated PEM group. In the nitrofen-exposed groups, however, there was an increase in the GVP activity in medial and distal segments of fetal intestine from pups of dams fed the 24% casein diet, and a decrease in the proximal segment in the 6% casein group.

The AGP activity in the fetal intestines was reduced in the medial and distal segments of the intestine of fetuses of PEM dams. Toxin exposure tended to result in an increase in enzyme activity in the proximal and medial segment in the control group and a decrease in the PEM group. The drop in the PEM group also occurred in the distal segment. An interaction between diet and treatment was indicated, affecting proximal ($p < 0.05$), medial ($p < 0.05$), and distal ($p < 0.02$) segments.

DISCUSSION

The data demonstrate that the maternal protein deficit resulted in a decrease in body weight and intestinal weight and length in the fetus. Maternal nitrofen exposure resulted in a decrease in all three parameters in fetuses of dams fed the control diet. In the group fed the PEM diet, while all three appeared somewhat reduced, only the reduction in intestinal length was statistically significant.

The ratios of intestinal weight and length to body weight were calculated to determine whether the effect on the intestine differed from that on the body as a whole. The data indicate that intestinal weight is affected by diet more than is body weight. Intestinal length, while reduced in total, is not as severely affected.

A reduction in intestinal weight can be the consequence of a decrease in total length or of reduced weight per unit length. The reduced weight to length ratio in this work suggests that there is a "thinning" effect in the intestine consequent to the PEM diet. Examination of stained paraffin sections are compatible with this conclusion, showing reduced villus height, fewer villi, and, in medial and distal segments, a reduced total intestinal diameter. The combined effects of these changes are to reduce the total absorptive surface of the intestine and the surface relative to body size in PEM pups.

In the nitrofen-exposed groups, changes in intestinal weight and length, which occurred primarily in the control group, were apparently proportional to the changes in body weight. The effect of reduced VH and VMM in the distal section might be compensated for by the increased proximal VW and medial intestinal diameter. Overall, the data suggest that the toxin exposure had little if any effect on the amount of absorptive surface.

Maternal protein restriction has been shown previously to reduce lipid and protein uptake by the enterocytes; however, the protein restriction was more severe

TABLE IV. Effects of Nitrofen Exposure* in the Protein-Energy-Malnourished Fetal Rat on Specific Dipeptidase Activities in Various Segments of Intestine**

	Treatment[a]						
	Carrier only		Nitrofen exposure		Significance of difference [a]: P		
	24% casein	6% casein	24% casein	6% casein	D	N	I
n	12	12	12	13			
Proximal							
GLP	2.45 ± 0.26[a]	1.82 ± 0.18[a]	1.86 ± 0.19[a]	1.75 ± 0.23[a]	NS	NS	NS
GVP	1.89 ± 0.20[a]	1.91 ± 0.16[a]	2.13 ± 0.18[a]	1.25 ± 0.12[b]	<0.01	NS	<0.01
AGP	0.85 ± 0.11[a]	1.00 ± 0.11[b]	0.86 ± 0.05[a]	0.63 ± 0.04[c]	NS	<0.05	<0.05
Medial							
GLP	4.98 ± 0.50[a]	3.93 ± 0.29[b]	4.99 ± 0.26[a]	3.96 ± 0.29[b]	<0.01	NS	NS
GVP	3.74 ± 0.22[a]	3.55 ± 0.16[a]	4.31 ± 0.15[b]	3.32 ± 0.20[a]	<0.005	NS	<0.05
AGP	2.06 ± 0.12[a]	1.96 ± 0.25[ab]	2.36 ± 0.08[a]	1.60 ± 0.09[b]	<0.01	NS	<0.05
Distal							
GLP	6.04 ± 0.73[a]	3.25 ± 0.47[b]	5.87 ± 0.79[a]	4.14 ± 0.40[b]	<0.0001	NS	NS
GVP	4.72 ± 0.43[ab]	3.71 ± 0.34[a]	5.80 ± 0.71[b]	3.88 ± 0.16[a]	<0.005	NS	NS
AGP	2.51 ± 0.26[a]	2.18 ± 0.38[ac]	3.55 ± 0.18[b]	1.58 ± 0.24[c]	<0.001	NS	<0.02

*25 mg in oil/kg maternal body weight/day by gavage.
**Δ 0.D/min/mg protein. D, diet; N, nitrofen; I, interaction; NS, not significant.
[a]Data are mean ± SEM. Numbers in a row not sharing a common superscript letter are significantly different.

TABLE V. Effects of Nitrofen Exposure* in the Protein-Energy-Malnourished Rat on Fetal Intestinal Lactase Activity**

	Treatment[a]				Significance of difference[a]: P		
	Carrier only		Nitrofen exposure				
Grade	24% casein	6% casein	24% casein	6% casein	D	N	I
n	12	12	12	13			
Proximal	125.66 ± 7.19[a]	119.66 ± 6.19[a]	115.90 ± 8.61[a]	95.55 ± 4.89[b]	<0.05	0.02	NS
Medial	96.12 ± 7.80[a]	82.41 ± 4.45[ab]	92.75 ± 9.36[a]	70.87 ± 7.55[b]	<0.05	NS	NS
Distal	30.74 ± 2.85[a]	14.29 ± 1.03[b]	23.85 ± 3.00[c]	14.05 ± 1.70[b]	<0.0001	NS	NS

*25 mg in oil/kg maternal body weight/day by gavage.

**μmoles glucose liberated/min/g protein. D, diet; N, nitrofen; I, interaction; NS, not significant.

[a]Data are mean ± SEM. Numbers in a row not sharing a common superscript letter are significantly different.

than that used in this study [34]. Histological study of absorption of macromolecules in the animals in this study indicates that neither maternal diet nor toxin exposure affected protein absorption. Newborn rats are known to be able to absorb intact protein by pinocytosis [35,36], but the major significance postnatally may be not nutritional, but immunological [37,38]. The reduced lipid absorption in the fetuses of PEM dams exposed to nitrofen may have some nutritional significance, since rat milk is high in fat and fat is the major energy source to the suckling rat.

Lactose digestion occurs largely in the upper portion of the small intestine. Thus, the reduction in intestinal lactase activity in the young of PEM dams may have some physiological significance postnatally (Table V).

Dipeptidase activity is normally highest in the medial and distal segments of the rat intestine [19]. The reduction in dipeptidase activity in these same segments in young of PEM dams suggests that these young are handicapped in their ability to digest and absorb dipeptides. Maternal nitrofen exposure, singly, appears to have little effect; however, the statistical analysis suggests an interaction between maternal PEM and toxin exposure, affecting AGP and possibly GVP activity.

Overall, it seems clear that maternal PEM has a marked effect on fetal intestinal size, morphology, and function. Maternal nitrofen exposure at the dosage used has little effect and little interaction with the nutritional deficit. In the rat, maternal nitrofen exposure at 12.5 and 25 mg/kg/day given on days 7–15 of gestation resulted in a reduction in fetal weight in a dose-related fashion [39]. Postnatal growth and survival rate are also reduced [40]. Although maternal nitrofen exposure might influence postnatal growth and development by its effects on intestinal morphology, these effects are sufficiently mild that they are unlikely to make a significant contribution to postnatal mortality rate in pups of either adequately nourished or malnourished dams.

REFERENCES

1. Page NP, Weisburger EK, Garner FM: "Bioassay of nitrofen for possible carcinogenicity." National Cancer Institute, Carcinogenesis, Technical Report Series CAS NO 1836-75-5, NCT-CG-TR-184:1, 1979.
2. Lambert R, Kunert JJ, Boger P: On the phytotoxic mode of action of nitrofen. Pestic Biochem Physiol II:267–274, 1979.
3. Ambrose AM, Larson PS, Borzelleca JF, Smith RB, Hennigar GR: Toxicological studies on 2,4 dichloro-p-nitrophenyl ether. Toxicol Appl Pharmacol 19:263–275, 1971.
4. Kimbrough RD, Gaines TB, Linder RE: 2,4-dichloro-4'-nitrodiphenyl ether. Arch Environ Health 28:316–328, 1974.
5. Stone LC, Manson JM: Effects of the herbicide 2,4-dichlorphenyl-p-nitrophenyl ether (nitrofen) on fetal lung development in rats. Toxicology 20:195–207, 1981.
6. Costlow RD, Manson JM: The heart and diaphragm: Target organs in the neonatal death induced by nitrofer (2,4,dichlorophenyl-p-nitrophenyl ether). Toxicology 20:209–227, 1981.
7. Chernoff N, Kavlock RJ: An in vivo teratology screen utilizing mice. J Toxicol Environ Health 10:541–550, 1982.
8. Gray LE Jr, Kavlock RJ, Chernoff N, Ferrell J, McLamb J, Ostby J: Prenatal exposure to the herbicide NIT destroys the rodent Harderian gland. Science 215:293–294, 1982.
9. Gray LE, Kavlock RJ, Chernoff N, Ostby J, Ferrell J: Postnatal developmental alterations following prenatal exposure to the herbicide 2,4,dichlorophenyl ether. A dose-response evaluation in the mouse. Toxicol Appl Pharmacol 67:1–14, 1983.
10. Kavlock RJ, Gray LE: Postnatal evaluation of morphological and functional effects of prenatal exposure to nitrofen in Long-Evans rat. J Toxicol Envir Health 11:679–690, 1983.

11. Gray LE, Kavlock RJ: The effects of herbicide 2,4 dichlorophenyl-p-nitrophenyl ether (NIT) on serum thyroid hormones in adult female mice. Toxicol Lett 15:231–235, 1983.
12. Manson JM, Brown T, Baldwin DM: Teratogenicity of nitrofen (2,4-dichloro-4′-nitrophenyl ether) and its effects on thyroid function in the rat. Toxicol Appl Pharmacol 73:323–325, 1984.
13. Porter WP, Hinsdill R, Fairbrother A, Olson LJ, Jaeger J, Yuill T, Bisgaard S, Hunter WG, Nolan K: Toxicant-disease-environment interactions associated with suppression of immune system, growth, and reproduction. Science 224:1014–1017, 1984.
14. Boyd EM: "Protein Deficiency and Pesticide Toxicity." Springfield: Charles C Thomas, 1982.
15. Boyd EM, Krupa V: Protein-deficient diet and diuron toxicity. Agric Food Chem 18:1104–1107, 1970.
16. Boyd EM, Krijnen CJ: Toxicity of captan and protein-deficient diet. J Clin Pharmacol 8:225–234, 1970.
17. Mayura D, Hayes AW, Berndt WO: Effect of dietary protein on teratogenicity of ochratoxin A in rats. Toxicology 27:147–157, 1983.
18. Shrader RE, Zeman FJ: Effect of protein deprivation on morphological and enzymatic development of neonatal rat tissue. J Nutr 99:401–412, 1969.
19. Mahboob S, Zeman FJ: Intestinal dipeptidase in the intestine of the prenatally protein-deprived rat. Nutr Repts Internatl 14:423–438, 1976.
20. Mahboob S, Zeman FJ: Lactase levels in the intestines of the young of protein-deficient rats. Nutr Repts Int 15:451–457, 1977.
21. Loh K-R, Shrader RE, Zeman FJ: Effect of maternal protein deprivation on neonatal intestinal absorption. J Nutr 101:1663–1672, 1971.
22. Zeman FJ, Fratzke ML: Protein, dipeptide and amino acid absorption in the young of protein-deprived rats. Pediatr Res 11:972–977, 1977.
23. Zeman FJ: Effect on the young rat of maternal protein restriction. J Nutr 93:167–173, 1967.
24. Shrader RE, Ferlatte MI, Zeman FJ: Early postnatal development of the intestine in progeny of protein-deprived rats. Biol Neonate 31:181–198, 1977.
25. Winer BJ: Appendix B, Table B5, Arcsin transformation In: "Statistical Principles in Experimental Design." New York: McGraw-Hill, 1962, p 650.
26. Siegel S: "Nonparametric Statistics for the Behavioral Sciences." New York: McGraw-Hill, 1956, pp 116–126.
27. Cornell R, Padykula HA: A cytological study of intestinal absorption in the suckling rat. Am J Anat 125:291–316, 1969.
28. Schneeberger-Keeley EE, Karnovsky MJ: Ultra-structural basis of alveolar capillary membrane permeability to peroxidase used as a tracer. J Cell Biol 37:781–793, 1968.
29. Josefsson L, Lindberg T: Intestinal dipeptidase I. Spectrophotometric determination and characterization of dipeptidase activity in pig intestinal mucosa. Biochim Biophys Acta 105:149–161, 1965.
30. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ: Protein measurement with the Folin phenol reagent. J Biol Chem 193:265–275, 1951.
31. Dahlqvist A: Assay of intestinal disaccharidases. Anal Biochem 22:99–107, 1968.
32. Ray AA, ed.: "SAS User's Guide." Cary, NC: SAS Institute, 1982.
33. Snedecor GW, Cochran WG: "Statistical Methods", 6th Ed. Ames IA: Iowa State University Press, 1971.
34. Shrader RE, Ferlatte MI, Zeman FJ: Early postnatal development of the intestine in the progeny of protein-deprived rats. Biol Neonate 31:181–198, 1977.
35. Clarke RM, Hardy RN: The use of [125 I]polyvinyl pyrrolidone K.60 in the quantitative assessment of the uptake of macromolecular substances by the intestine of the young rat. J Physiol (Lond) 204:113–125, 1969.
36. Clarke RM, Hardy RN: An analysis of the mechanism of cessation of uptake of macromolecular substances by the intestine of the young rat ("Closure"). J Physiol (Lond) 204:127–134, 1969.
37. Halliday R: The absorption of antibodies from immune sera by the gut of the young rat. Proc R Soc Lond [Biol] 143:408–413, 1955.
38. Halliday R: Prenatal and postnatal transmission of passive immunity to young rats. Proc R Soc Lond [Biol] 144:427–430, 1955.
39. Kavlock RJ, Chernoff N, Rogers E, Whitehouse D, Carver B, Gray J, Robinson K: An analysis of fetotoxicity using biochemical endpoints of organ differentiation. Teratology 26:183–194, 1982.
40. Kavlock RJ, Gray JA: Morphometric, biochemical and physiological assessment of perinatally induced renal dysfunction. J Toxicol Environ Health 11:1–13, 1983.